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Molecular characterization and susceptibility screening for methicillin-resistant *Staphylococcus aureus* reveals the dominant clones in a tertiary care hospital in Al Qassim, Saudi Arabia

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ABSTRACT

Objective: *Staphylococcus aureus* has become an important pathogen in hospitals worldwide. Despite its differentiation into human and animal lineages, common methods are used for genotyping. While these methods are useful, they are based on the stable genome, and hence, are insensitive to host-specific subtyping. The objectives of this study were to investigate the repeat-domain of the Clumping-Factor A gene (*clfA*-R) as an objective and adaptation-sensitive approach.

Methodology: We have used 113 isolates for susceptibility testing and genotyping by polymerase chain reaction amplification of the *clfA*-R regions. Of these, 105 were from King Fahad Specialist Hospital, Buraidah and eight were published sequences used as references. Isolates were further confirmed as *S. aureus* by the commercial Kits. Amplicon sizes were measured and the number of the 18-bp-repeating-units in each isolate was determined against that of methicillin-resistant *S. aureus* COL (MRSA) sequence.

Results: Results showed that all 42 nasal screening isolates (100%) and all but six isolates from clinical specimens were MRSA with 37% of the former and 50% of the latter isolates showing community-acquired-MRSA susceptibility patterns. *clfA*-R analysis grouped 113 isolates into 14 repeat-genotypes. The two dominant types, D and X, represented the long- and short *clfA*-R types found in humans and animals, respectively. Linezolid, rifampicin, and vancomycin were the drugs of choice.

Conclusions: *clfA*-R was useful in rapid genotyping and implied host-specific phenotypic properties of the *ClfA*. It has been recommended that the approach used in regional laboratories for uniform strain-profiling. Future work will show more insights into the gene content and origins of clones.

Keywords: Molecular epidemiology, molecular typing, methicillin-resistant *Staphylococcus aureus* screening, *Staphylococcus aureus*

Introduction

Despite enormous efforts, *Staphylococcus aureus* continued to emerge causing outbreaks and posing significant clinical challenges in hospitals worldwide. Of particular concern is the devastating form of septicemia-pyaemia first described by Ogston[1] and still results in high rates of morbidity and mortality.[2] Through centuries, this pathogen has survived huge advances in medical care and adapted to become the leading cause of skin and soft tissue infections today.[3,4] A unique property of *S. aureus* is the possession of novel and highly advanced mechanisms of adaptation, in a clonal genome, that enables the emergence of host-associated strains. Although it has been well established that *S. aureus* has a stable genome based on house-keeping and highly polymorphic genes, new strains continued to emerge in different hosts.[5,6] For instance, several countries in the region reported recent emergences of novel methicillin-resistant *S. aureus* (MRSA) strains demonstrating an expanding MRSA repertoire. These included the emergence of previously unreported clonal complexes and novel strains.[7-10] Thus, since its appearance in the early 1960s,[11] hospital-associated-MRSA incidence steadily increased accounting for over 60% of the species isolates in US hospitals alone by the year 2004 according to the National Nosocomial Infection Surveillance system data (2004).[12] The annual hospitalization rate of 125,969 was

reported for *S. aureus* with mortality rates similar to that of AIDS, tuberculosis, and viral hepatitis combined.[13,14] This was followed by the most alarming incidence in MRSA history, the emergence of community acquired-MRSA (CA-MRSA) lineage as a primary cause of skin, soft tissue, and bloodstream infections in young healthy adults and children with no prior health-care exposure.[15,16] Since then, decades of global outbreaks of CA-MRSA lineages, including USA300 and USA400 were reported.[17,18] Furthermore, the emergence of human pandemic clones originated in bovine mastitis and other livestock posed significant risks to public health.[19-22] We have surveyed Al-Qassim regional hospital and identified many multi-resistant bacterial pathogens. Among them, *S. aureus* was one of the most resistant isolates found with patterns typical of CA-MRSA clones.[23]

While significant resources and time were given to basic research on human *S. aureus*, little attention was placed on the development of sensitive and host-specific typing and detection systems. The genome-based typing markers, although useful as gold standards, are often limited by their low discriminatory powers toward newly emerging subtypes in different hosts. This is mainly due to the clonality of *S. aureus* genome from different host species. For example, pathogenic strains from humans and animals showed the same genetic background and gene content.[24,25] Similarly, there was no genomic diversity between commensal and pathogenic strains in a comparative genome study.[26] Thus, the common genetic background allowed DNA transfer between host-specialized lineages.[27,28] Consequently, acquisitions of new factors triggered significant global transcriptome shifts leading to the evolution of CA-MRSA lineages. These were characterized by panton-valentine leukocidin toxin and SCCmec type IV element and susceptibility to non-β-lactam antibiotics.[29-31] While the acquisition of elements played a role in virulence, it does not explain the overall enhanced virulence *per se*.[18,32,33] For these reasons genome-based molecular typing markers, although useful as gold standards, are often limited by low sensitivity toward new subtypes. Alternatively, intrinsic, constitutive, and universal markers that are strategically located on cell surfaces of bacterial species can be useful candidates in objectively sub-typing host-specific lineages.

It has been well established that the repeat-rich adaptation-sensitive surface proteins play major roles in adaptation. Repeat based markers are often superior to multilocus sequence typing in the detection of polymorphism among *S. aureus* isolates.[34] For example, Clumping Factor B (*clf*B) showed congruence with SCCmec and was a promising alternative to pulsed-field gel electrophoresis when used in a double-locus typing with spa, another repeat-based typing system.[35] However, *ClfB* biological activity is limited only to log phase under increased oxygenation because it is digested by the stationary phase proteases.[36-38] In contrast, clumping-factor A (*clfA*) is constitutively expressed under host environments. Moreover, although SCCmec typing has been widely used, its mobility and the appearances of invasive methicillin-sensitive *S. aureus* (MSSA)[39] made it less useful than intrinsic typing markers.

The objectives of the present work were to first identify MRSA phenotypes from clinical specimens and screening procedures and to genotype resulting MRSA isolates using the R domain of the *clfA* gene.

Methodology

Ethical statement

Although this study used only bacterial isolates, and hence, would be exempted from criteria under Human Subject Research, ethical approval for this project was obtained from the Ethical Committee of the Ministry of Health, General Health Affairs Directorate, Training, Medical Education and Research, Al Qassim Province, no. 687/44/45 and No 688/44/45 to fully comply with the request of ethical clearance.

Bacterial strains, sequences, and susceptibility testing

A total of 113 MRSA isolates and strains were used in this study for genotyping by R-domain of the *clfA* gene. Eight isolates were published genome sequences of major strains [Table 1], but the majority of isolates (105) were from King Fahad Specialist Hospital (KFSH) [Tables 2a,b and 3], Buraidah, KSA. The KFSH in Buraidah, Al-Qassim Province is a 540-bed tertiary care center that receives patients from all socioeconomic strata within Al-Qassim Province of about 1 million in population size. The 105 isolates used in this study were both from routine nasal MRSA screening procedures using the more stable agent cefoxitin carried out on patient admission as well as from clinical specimens submitted to the microbiology laboratory department for routine diagnosis and antimicrobial susceptibility testing. Isolates were first identified through routine standard bacteriological methods and inoculation of an automated system for rapid identification (ID) and antimicrobial susceptibility testing. The ID and susceptibility testing were done using automated Microscan according to the standard recommendations. This was followed by disc diffusion testing against oxacillin and susceptibility to other non-beta-lactams as possible indicators for CA-MRSA. Interpretations were based on the Clinical and Laboratory Standards Institute (2012)[40] guidelines.

Isolates were confirmed as *S. aureus* using a specific detection kit (*S. aureus* Detection Kit 29300 Norgen Biotek, Thorold, ON, Canada). All isolates were from different specimens. Antimicrobial susceptibility testing was carried out according to Said *et al*., (2014).[23] In addition, published sequences of 8 strains (mastitis strains RF122, MRSA252, MRSA COL, MSSA 476, N315, MW2, Mu50, and USA300) were used as references for analysis. Glycerol stocks of isolates in trypticase soy broth (TSB) were stored at −80°C.

Table 1: Numbers, names, addresses, and sources of isolates and sequenced strains used in the clfA-R-domain based genotyping of Methicillin resistant *Staphylococcus aureus*

No.	Strain/isolate	Source	Site	Origin	*ClfA* repeat copy number and genotype
105	Methicillin resistant *Staphylococcus aureus* isolate	Human	King Fahad Specialist Hospital, Buraidah	Human	Table 3
***clfA*-R domain from published sequences of strains**					
1	*S. aureus* subsp. aureus COL (MRSA)	TIGR (J. Craige Venter Institute)	http://www.tigr.org	Human	50.5=genotype C
2	*S. aureus* subsp. aureus MRSA252 (HA-MRSA)	Welcome Trust Sanger Institute	http://www.sanger.ac.uk/	Human	66=genotype A
3	*S. aureus* subsp. aureus N315 HA-MRSA	Juntendo University	http://www.staphylococcus.org/jp/	Human	59.5=genotype A
4	*S. aureus* subsp. aureus MSSA476 (CA-MSSA) hypervirulent	Welcome Trust Sanger Institute	http://www.sanger.ac.uk/	Human	49=genotype C
5	*S. aureus* subsp. aureus MW2 (CA-MRSA)	NITE	http://www.bio.nite.go.jp/	Human	51=genotype C
6	*S. aureus* subsp. aureus Mu50 MRSA–VRSA	Juntendo University	http://www.staphylococcus.org/jp/	Human	49.5=genotype C
7	*S. aureus* subsp. aureus USA300 strong association with unusually invasive disease (invasive MRSA)	University of California, San Francisco	ttp://www.ncbi.nlm.nih.gov/sites/entrez?db=genomeprj&cmd=Retrieve&dopt=Overview&list_uids=16313	Human	49=genotype C
8	Mastitis *S. aureus* RF122	University of Minnesota	http://www.ncbi.nlm.nih.gov/sites/entrez?db=genomeprj&cmd=Retrieve&dopt=Overview&list_uids=63	Bovine mastitis	46=genotype Q

MRSA: Methicillin-resistant *S. aureus*, MSSA: Methicillin-sensitive *S. aureus*, *S. aureus*: *Staphylococcus aureus*

Genomic DNA extraction

All isolates were refreshed and grown for 18 h in Luria-Bertani and TSB media. Genomic DNA extractions were carried out according to the manufacturer's protocol for Bacterial Genomic DNA Isolation (Kit # 17900 Norgen Biotek, Thorold, ON, Canada). For efficient yield, *S. aureus* isolates were pre-digested with lysostaphin (Sigma) and lysozyme (Sigma) to break cell walls and render extraction of nucleic acids easy. Isolated genomic DNA was quantitated and either used immediately after quantitation or stored at –20°C for a short time or at –80°C for long storage period until used.

Polymerase chain reaction (PCR) amplification and electrophoresis

All of the isolates were tested to confirm species identity and the presence of the *clfA* gene with intact repeat regions. Several optimization experiments were carried out to set up template amount, primer locations, gel systems, software, and documentation. In the final round of amplification, high-quality gel resolutions were obtained in PCR products amplified in a series of gels 1–8. The representative Figure 1 shows the indicated gel picture with isolates from 26 to 105 (Lanes 26–105). On this figure, the sizes of amplicons are shown above the DNA bands, and the corresponding repeat copy numbers are shown below the bands.

PCR amplification of *clfA* R-domain typing was carried out as described previously with some modifications.[41,42] Briefly, Techne Thermal Cycler PCR #TC-412 was used for amplification of the R-domains from the *clfA* gene of all isolates using specific (desalted) primers pairs as follows: Forward primer, cf-F TCCTGAACAACCTGATGAGC and Reverse primer, cf-R AGGTGAATTAGGCGGAACTAC (Invitrogen Life Technologies, Europe). The PCR conditions used were: Initial denaturation at 94 for 4 min followed by 30 cycles each of denaturation for 1 min at 94°C, annealing at 58°C for 1 min, and extension at 72°C for 1 min, and final extension for 1 min at 72°C. A final hold at 10°C was added, but all samples were removed immediately after finishing. Agarose (Invitrogen Inc) gel electrophoresis of PCR amplicons was carried out in a Bio-Rad Sub-Cell for submerged horizontal electrophoresis (15 cm × 25 cm; 15-well combs) and in 1 litter base buffer volumes in the tanks connected to Bio-Rad PowerPac 200 electrophoresis power supply. After electrophoresis, ethidium bromides (Invitrogen Inc.) stained gels were illuminated and gel pictures were analyzed in the gel photo-doc system using the associated software.

clfA-R domain-based genotyping

PCR amplicons sizes on gels were measured against the 100 bp makers (Invitrogen Inc). The amplicon sizes were shown (bp) on the gel band, and its estimated corresponding copy number was indicated below the band. Because primers flanked a stable location on *S. aureus* genome sequence, differences in product sizes were expressed as differences in R-domain copy numbers

Table 2a: Antimicrobial susceptibility test, amplified *clfA* R-domain sizes, the corresponding repeat copy numbers and associated genotypes of methicillin-resistant *S. aureus* isolated from MRSA screens and clinical specimens at King Fahad Specialist Hospital, Buraidah

Gel	Isolate No.	Specmen site	Cefoxitin nasal screening	Clindamycin	Ciprofloxacin	Erythromycin	Gentamycin	Linezolid	Oxacillin	Pen blac	Rifampin	Tetracyclin	Trim/ SUL	Vancomycin	MRSA/ MSSA	*clfA*-Repeat genotype (RT)‡	*clfA*-Repeat copy number	Total *clfA*-R-domain size
1	1	Wound	Positive	S	S	S	S	S	R	Positive	S	S	S	S	MRSA	**X**	**52**	**1000**
	2	Wound	Positive	S	S	S	S	S	R	Positive	S	S	S	S	MRSA	Q	45	850
	3	Wound	Positive	R	R	R	S	S	R	Positive	S	R	R	S	MRSA	**X**	**52**	**1000**
	4	Trch-ASP	Positive	S	S	S	S	S	R	Positive	S	S	S	S	MRSA	**X**	**52**	**1000**
	+																	
	6	Wound	Positive	R	R	R	R	S	R	Positive	S	R	R	S	MRSA	A	65	1250
	+																	
	8	Trch ASP	Positive	R	S	R	S	S	R	Positive	S	R	S	S	MRSA	**X**	**52**	**1000**
	*																	
	10	Wound	Positive	S	S	S	R	S	R	Positive	S	S	S	S	MRSA	E	55	1050
	11	Trch ASP	Positive	R	S	R	S	S	R	Positive	S	R	S	S	MRSA	**X**	**52**	**1000**
	12	Wound	Positive	R	R	R	S	S	R	Positive	S	R	R	S	MRSA	B	60	1150
	13	Ear	Positive	S	S	S	S	S	R	Positive	S	S	S	S	MRSA	B	60	1150
2	14	Wound	Positive	S	R	S	R	S	R	Positive	S	S	S	S	MRSA	**D**	**57**	**1100**
	*																	
	16	Wound	Positive	R	R	R	R	S	R	Positive	S	R	R	S	MRSA	**X**	**52**	**1000**
	17	Wound	Positive	R	S	R	S	S	R	Positive	S	R	R	S	MRSA	**X**	**52**	**1000**
	18	Wound	Positive	R	R	R	R	S	R	Positive	S	R	R	S	MRSA	**X**	**52**	**1000**
	*																	
	23	Trch ASP	Positive	S	S	S	S	S	R	Positive	S	S	S	S	MRSA	Q	47*	900
	24	Blood	Positive	R	R	R	R	S	R	Positive	S	R	NA	S	MRSA	Q	47*	900
	25	Wound	Positive	R	R	R	S	S	R	Positive	S	R	NA	S	MRSA	Q	47*	900
3	26	Wound	Positive	R	R	R	S	S	R	Positive	S	R	NA	S	MRSA	E	55	1050
	27	Blood	Positive	R	R	R	I	S	R	Positive	R	S	R	S	MRSA	**D**	**57**	**1100**
	28	Wound	Positive	S	S	S	S	S	R	Positive	S	R	NA	S	MRSA	E	55	1050
	29	Wound	Positive	S	S	S	S	S	R	Positive	S	R	S	S	MRSA	**D**	**57**	**1100**
	30	Wound	Positive	R	R	R	R	S	R	Positive	S	R	R	S	MRSA	E	55	1050
	31	Wound	Positive	S	S	S	S	S	R	Positive	S	S	S	S	MRSA	**D**	**57**	**1100**
	32	Wound	Positive	S	S	S	S	S	R	Positive	S	S	S	S	MRSA	F	49	950
	33	Wound	Positive	R	S	R	S	S	R	Positive	S	S	S	S	MRSA	F	49	950
	34	Trch ASP	Positive	R	S	R	S	S	R	Positive	S	R	S	S	MRSA	E	55	1050
	35	Trch ASP	Positive	R	R	R	R	S	R	Positive	R	R	R	S	MRSA	E	55	1050
	36	Wound	Positive	S	S	S	S	S	R	Positive	S	R	S	S	MRSA	E	55	1050
	37	Wound	Positive	S	S	S	S	S	R	Positive	S	S	S	S	MRSA	**D**	**57**	**1100**
	+																	
	7	Blood	Positive	R	S	R	S	S	R	Positive	S	R	S	S	MRSA	E	55	1050
	39	Wound	Positive	S	S	S	S	S	R	Positive	S	S	S	S	MRSA	E	55	1050
4	40	Wound	Positive	R	R	R	S	S	R	Positive	S	R	R	S	MRSA	E	55	1050
	41	Wound	Negative	S	S	S	S	S	S	Positive	S	S	S	S	MSSA	B	60	1150

(*Contd...*)

Table 2a: (*Continued*)

Gel	Isolate No.	Specmen site	Cefoxitin nasal screening	Clindamycin	Ciprofloxacin	Erythromycin	Gentamycin	Linezolid	Oxacillin	Pen blac	Rifampin	Tetracyclin	Trim/ SUL	Vancomycin	MRSA/ MSSA	*clfA*-Repeat genotype (RT)‡	*clfA*-Repeat copy number	Total *clfA*-R-domain size
	42	Wound	Positive	S	S	S	S	S	R	Positive	S	S	S	S	MRSA	**X**	**52**	**1000**
	43	Wound	Positive	R	R	R	R	S	R	Positive	S	R	R	S	MRSA	B	60	1150
	44	Wound	Positive	S	S	S	S	S	R	Positive	S	S	S	S	MRSA	**D**	**57**	**1100**
	45	Blood	Positive	S	S	S	S	S	R	Positive	S	S	S	S	MRSA	**X**	**52**	**1000**
	46	Ear	Negative	R	S	R	S	S	S	Positive	S	S	S	S	MSSA	**D**	**57**	**1100**
	47	Ear	Negative	S	S	S	S	S	S	Positive	S	R	S	S	MSSA	**D**	**57**	**1100**
	48	Ear	Negative	S	S	S	S	S	S	Positive	S	S	S	S	MSSA	**X**	**52**	**1000**
	*																	
	50	Trch ASP	Positive	S	R	S	R	S	R	Positive	S	R	S	S	MRSA	**D**	**57**	**1100**
	51	Ear	Negative	S	S	S	S	S	S	Positive	S	R	S	S	MSSA	Q	45*	850
5	52	Wound	Positive	R	S	R	S	S	R	Positive	S	S	S	S	MRSA	**X**	**52**	**1000**
	53	Wound	Positive	S	S	S	R	S	R	Positive	S	R	S	S	MRSA	Q	45*	850
	*																	
	56	Wound	Positive	R	R	R	S	S	R	Positive	S	R	R	S	MRSA	**D**	**57**	**1100**
	57	Wound	Positive	R	R	R	R	S	R	Positive	S	R	R	S	MRSA	**X**	**52**	**1000**
	58	Trch ASP	Negatve	S	S	S	S	S	S	Positive	S	S	S	S	MSSA	F	49	950
	59	Blood	Positive	R	R	R	R	S	R	Positive	R	R	R	S	MRSA	F	49	950
	60	Wound	Positive	R	R	R	S	S	R	Positive	S	R	R	S	MRSA	B	60	1150
	5	Wound	Positive	R	R	R	R	S	R	Positive	S	R	R	S	MRSA	**X**	**52**	**1000**
	38	Wound	Positive	R	R	R	S	S	R	Positive	S	R	R	S	MRSA	G	21	400
	61	Wound	Positive	R	R	R	S	S	R	Positive	S	R	R	S	MRSA	B	60	1150
	62	Wound	Positive	R	S	R	S	S	R	Positive	S	S	S	S	**MRSA**	**D**	**57**	**1100**
	63	Screening	Positive	R	R	R	R	S	R	Positive	S	R	R	S	MRSA	B	60	1150

‡(X=52, C=50±1, Q=45±1) *Isolates missed; +Isolates re-run on a different lane on the gels. Bolded antibiotics=drugs of choice. MRSA: Methicillin-resistant *S. aureus*, MSSA: Methicillin sensitive *S. aureus*, *S. aureus*: *Staphylococcus aureus*, *clfA*: Clumping-factor A

Table 2b: Antimicrobial susceptibility test, amplified *clfA* R-domain sizes, the corresponding copy numbers and associated genotypes of methicillin-resistant *Staphylococcus aureus* recovered from MRSA screening at King Fahad Specialist Hospital, Buraidah

Gel	Isolate no	Specmen site	Cefoxitin nasal screening	Clindamycin	Ciprofloxacin	Erythromycin	Gentamycin	Linezolid	Oxacillin	Pen blac	Rifampin	Tetracyclin	Trim/ sul	Vancomycin	MRSA/ MSSA	*clfA*-Repeat genotype (RT)‡	*clfA*-Repeat copy number	Total *clfA*-R-domain size (bp)
6	64	Screening	Positive	R	R	R	R	S	R	Positive	S	R	R	S	MRSA	**D**	**57**	**1100**
	65	Screening	Positive	R	R	R	S	S	R	Positive	S	S	S	S	MRSA	**D**	**57**	**1100**
	66	Screening	Positive	S	S	S	S	S	R	Positive	S	S	S	S	MRSA	**D**	**57**	**1100**
	67	Screening	Positive	S	S	S	S	S	R	Positive	S	S	S	S	MRSA	**D**	**57**	**1100**
	68	Screening	Positive	S	S	S	S	S	R	Positive	S	S	S	S	MRSA	F	49	950
	*																	
	70	Screening	Positive	S	S	S	R	S	R	Positive	S	R	S	S	MRSA	**X**	**52**	**1000**
	71	Screening	Positive	S	S	S	S	S	R	Positive	S	S	S	S	MRSA	**D**	**57**	**1100**
	72	Screening	Positive	S	S	S	S	S	R	Positive	S	R	S	S	MRSA	**D**	**57**	**1100**
	73	Screening	Positive	R	R	R	R	S	R	Positive	S	I	R	S	MRSA	F	49	950
	74	Screening	Positive	R	R	R	S	4	R	Positive	S	R	S	S	MRSA	H	31	600
	75	Screening	Positive	S	S	S	S	S	R	Positive	S	S	S	S	MRSA	F	49	950
	76	Screening	Positive	S	S	S	S	S	R	Positive	S	S	S	S	MRSA	**D**	**57**	**1100**
	77	Screening	Positive	R	S	R	S	S	R	Positive	S	S	S	S	MRSA	**X**	**52**	**1000**
7	78	Screening	Positive	S	S	S	S	S	R	Positive	S	S	S	S	MRSA	**B**	**60**	**1150**
	79	Screening	Positive	R	S	R	S	S	R	Positive	S	S	S	S	MRSA	**D**	**57**	**1100**
	80	Screening	Positive	R	R	R	R	S	R	Positive	S	R	R	S	MRSA	I	63	1200
	81	Screening	Positive	R	S	R	S	S	R	Positive	S	S	S	S	MRSA	**D**	**57**	**1100**
	82	Screening	Positive	S	S	S	R	S	R	Positive	S	S	S	S	MRSA	**D**	**57**	**1100**
	83	Screening	Positive	S	S	S	S	S	R	Positive	S	S	S	S	MRSA	**B**	**60**	**1150**
	84	Screening	Positive	R	R	R	R	S	R	Positive	R	R	R	S	MRSA	**B**	**60**	**1150**
	85	Screening	Positive	R	S	R	S	S	R	Positive	S	S	S	S	MRSA	J	42	800
	86	Screening	Positive	R	R	R	R	S	R	Positive	S	S	S	S	MRSA	Q	47*	900
	87	Screening	Positive	R	R	R	R	S	R	Positive	S	R	R	S	MRSA	**X**	**52**	**1000**
	88	Screening	Positive	S	S	S	S	S	R	Positive	S	S	S	S	MRSA	**D**	**57**	**1100**
	89	Screening	Positive	R	R	R	R	S	R	Positive	S	R	R	S	MRSA	**D**	**57**	**1100**
	90	Screening	Positive	S	S	S	S	S	R	Positive	S	R	S	S	MRSA	**X**	**52**	**1000**
	91	Screening	Positive	S	S	S	S	S	R	Positive	S	S	S	S	MRSA	**X**	**52**	**1000**
8	92	Screening	Positive	R	R	R	R	S	R	Positive	S	R	R	S	MRSA	K	53	1020
	93	Screening	Positive	R	R	R	R	S	R	Positive	S	R	R	S	MRSA	K	53	1020
	94	Screening	Positive	S	S	S	S	S	R	Positive	S	S	S	S	MRSA	K	53	1020
	95	Screening	Positive	R	R	R	R	S	R	Positive	S	R	R	S	MRSA	F	49	950
	96	Screening	Positive	R	S	R	S	S	R	Positive	S	S	S	S	MRSA	C	51	980
	97	Screening	Positive	S	S	S	S	S	R	Positive	S	S	S	S	MRSA	Q	47*	900
	98	Screening	Positive	R	R	R	R	S	R	Positive	R	R	R	S	MRSA	K	53	1020
	99	Screening	Positive	R	R	R	R	S	R	Positive	S	R	R	S	MRSA	C	51	980
	100	Screening	Positive	S	S	S	S	S	R	Positive	S	S	S	S	MRSA	L	23	450
	101	Screening	Positive	S	S	S	R	S	R	Positive	S	S	S	S	MRSA	B	60	1150
	102	Screening	Positive	S	S	S	S	S	R	Positive	S	S	S	S	MRSA	E	55	1050
	103	Screening	Positive	S	S	S	R	S	R	Positive	S	S	S	S	MRSA	B	60	1150
	104	Screening	Positive	S	S	S	R	S	R	Positive	S	S	S	S	MRSA	B	60	1150
	105	Screening	Positive	S	S	S	R	S	R	Positive	S	S	S	S	MRSA	**D**	**57**	**1100**

‡(X=52, C=50±1, Q=45±1) *Isolates missed; + Isolates re-run on a different lane on the gels. Bolded antibiotics=drugs of choice MRSA: Methicillin-resistant *S. aureus*, MSSA: Methicillin sensitive *S. aureus*, *S. aureus*: *Staphylococcus aureus*, *clfA*: Clumping-factor A

Table 3: Number and dominant *clfA* genotypes of methicillin *Staphylococcus aureus* isolated from MRSA screening and clinical specimens at King Fahad Specialist Hospital, Buraidah, KSA

No of *clfA*-types	Repeat Type (RT)* (X=52, C=50, Q=45±1)	Number of isolates in each genotype	Index of discrimination
14 Genotypes A-L and Q to X	D	24	0.9
	X	19	
	B	13	
	E	11	
	Q	8	
	F	8	
	K	4	
	C	2	
	H	1	
	I	1	
	J	1	
	L	1	
	A	1	
	G	1	

clfA: Clumping-factor A

of different isolates. Primers were designed on *S. aureus* COL genome sequence and its *clfA* R-domain length, i.e., number of repeat units, was used as a reference number for inferring copy numbers in other isolates. In *S. aureus* COL sequence, an amplicon size of 960 bp was obtained that corresponded to 50.5 copies of repeating 18 bp units in the R-domain. Accordingly, repeat types (RTs) of isolates were assigned on the basis of their differences from the reference by the 18-bp copies. For instance, two isolates with a difference in a single 18-bp copy were considered to be two different genotypes, i.e., different RTs. We first determined the dominant genotypes, and then we identified the variant RTs for *clfA* within the major groups to reach to dominant clones.

Discriminatory power of *clfA* typing

Discriminatory power of *clfA* typing was carried out by determination of RTs based on differences in the copy number of the 18-bp tandem repeats within the R domain of the *clfA* gene. The online discriminatory power calculator (http://biophp.org/stats/discriminatory_power/demo.php) was used to calculate the index of discrimination (ID) of isolates [Table 2], where a value of one indicated that each isolate was different and a value of 0 indicated that all isolates were identical. The numerical index of discriminatory power was used to give numerical estimates for strain typing, and the values (defined as the average probability that the typing system will assign a different type to two unrelated strains randomly sampled in the microbial population of a given taxon) were estimated, as described previously.[43] We attempted to correlate relationships based on the combined genetic as well as biological differences, i.e., the repeat unit differences as a measure of protein polymorphism among isolates under different host conditions. This would allow a rapid and specific primary screening tool without the need for extensive sequencing.

Results

As shown in Table 3, all MRSA isolates identified in clinical specimens, except for six mostly from the ear, were also MRSA positive for nasal screening. The aforementioned six MSSA isolates were penicillin resistant but susceptible to semisynthetic penicillinase-stable penicillins, i.e., oxacillin and also to cefoxitin nasal screening and non-beta-lactam antibiotics. Table 2a also shows significant number of MRSA isolates (37%) susceptible to other non-beta-lactams. In addition, the nasal screening procedure of 42 patients [Table 2b] revealed a similar susceptibility pattern to that in Table 2a. These were 100% positive for MRSA, oxacillin, and penicillin with 50% showing the CA-MRSA susceptibility pattern. Antimicrobial susceptibility testing showed that linezolid, rifampicin, and vancomycin were still among the drugs of choice for the treatment of staphylococcal infections in the hospital.

clfa typing: *ClfA*-genotyping identified 14 RTs designated, A to L, and Q and X with a high discriminatory power of ID = 0.9 [Table 2]. The eight published strains [Table 1] belonged to three RTs, namely, A, C, and Q. The majority of the isolates belonged to the first six RTs. Based on the length of the R domain, two major clonal groups were identified, namely, (a) long *clfA*-R group included A and I with 65 and 63 copy numbers, respectively, that were represented by single isolates each. These were followed by types B (13 isolates and 60 copies), and D which were the major clone in this group with 24 isolates (22%) and had 57 repeat copies. The source of isolates in the long R domain groups were almost always from wound infections and nasal MRSA screening.

The second clonal group was the (b) short *clfA*-R group represented mainly by the type X which was the major clone

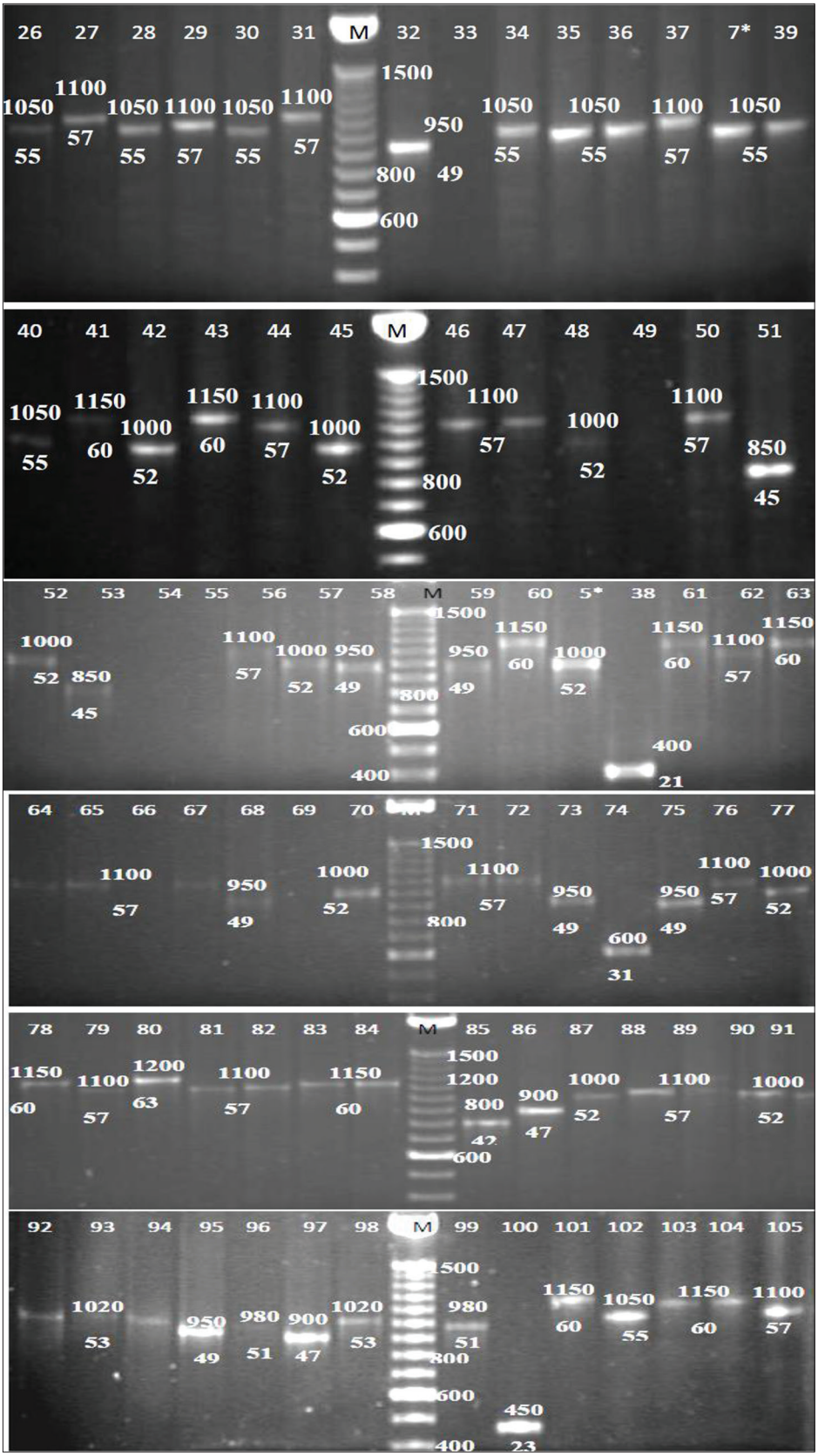

Figure 1: Representative gel pictures of polymerase chain reaction amplicons of the Clumping-Factor A-R domains from methicillin-resistant *Staphylococcus aureus* isolates (Lanes 26-51) recovered from screening and clinical specimens in King Fahad Specialist Hospital, Buraidah, Saudi Arabia. (Ethidium bromide illuminated, 1% agarose (Invitrogen Inc) gel picture in Bio-Rad Sub-Cell (15 cm × 25 cm; 15-well combs) tank for submerge electrophoreses in 1-litter base buffer volumes using Bio-Rad PowerPac 200 electrophoresis power supply)

in this group with 19 isolates (18%) each with 52 copies. The remaining RTs were quite variable and were usually represented by a few isolates. These were RT E (11 isolates, 10%), F and Q (8 isolates each, 7.6%), and K and C with 4 and 2 isolates, respectively. All other types had a single isolate each. This study also identified a few rare types with unusually smaller *clfA R* domains and much lower copy numbers than those of bovine lineages. These included genotypes G, H, J, and L with only 21, 31, 42, and 23 copies of repeats, respectively.

Discussion

This study investigated the usefulness of a PCR based genotyping and clonal differentiations of MRSA isolate using the repeat domain of the *clfA* gene as a marker. The finding that all isolates of nasal screening as well as clinical specimens [Table 2a and b], except for six, were MRSA positive correlates well with potential endogenous infections and MRSA carriage state. This agrees with the previous report that MRSA nares' swab was a more accurate predictor of MRSA wound infection compared with clinical risk factors in emergency department patients with skin and soft tissue infections.[4] However, the pattern similarity of the aforementioned six ears MSSA isolates to that of common CA-MRSA phenotypes, i.e., resistance to penicillin and susceptibility to non-beta-lactam antibiotics, strongly imply potential evolutionary dynamics of two lineages with two different mechanisms of invasiveness. This also indicates to the need for reducing the strong bias for sequencing only epidemic MRSA over MSSA strains. This practice has obscured the evolutionary lines of highly specialized MSSA populations.[44] Furthermore, in this study, significant rates of MRSA isolates from clinical specimens (37%) and screening (50%) showed the widely reported pattern of the CA-MRSA lineage [Table 2a and b].

The *clfA*-Repeat typing identified two major clonal groups based on their *clfA*-R domain lengths. These were named as "long *clfA*-R group" that included RTs A, I, B, and D. The latter was the major clone in this group with 24 isolates. The fact that this group was almost always recovered from nasal MRSA screening and wound infections implied their association with the host. These findings are consistent with the earlier reports where 21 of the 24 isolates from wound infections had the longest *cflA*-R domain.[41] It has been shown that variations in coding tandem repeat results in length polymorphism and in turn affect the function or antigenicity of the protein at that specific host.[45] The obtained phenotypic property of the marker is significant in the mechanisms of adaptation and colonization of a specific host site. In turn, exploitation of this structural property can be useful in host- and organ-specific typing for tracing the sources of infection.

The second clonal group was the "short *clfA*-R domain group" represented mainly by type X (19 isolates with 52 copies each) as the major clone in this group. The length of the *clfA*-R region in all these isolates was typical of those isolated from the bovine mastitis lineage of *S. aureus*[41,42] implying potential transmissions from the animal. Cross transmissions to humans through dairy and other animal products have been commonly reported in different geographic regions around the globe. This is further supported by the high prevalence rates of short RT (X and Q) only in clinical specimens from infections in contrast to those from the resident nasal carriage (long RTs D and B) recovered mainly from nares' screening. Using *clfA*-R domain typing, we have previously established that the existence of long and variable repeats in humans and the dominance of a clonal motif in bovine mastitis implied the occurrence of a specific selection in the bovine mammary gland. For these reasons, vigilance is needed for monitoring staphylococcal populations at the human-animal interface where major typing methods lack discriminatory power for host-specific differences.[46]

Conclusions

In this study, we have investigated the usefulness of the R domain of the *clfA* core adhesion gene in the rapid ID of MRSA clones and their host- and organ-specific phenotypic profiles of the marker. The majority of isolates fell into two dominant repeat-types, D and X that represented the long- and short *clfA*-R types found in humans and animals, respectively. Thus, *clfA*-R domain was useful in the differentiation of MRSA clones and implied phenotypic host-specificity inherent in the marker. It has also been recommended that *clfA-R* screening be a unified method in regional laboratories for quick ID and tracking of circulating clones. Future vertical genetic analysis will show more insights into the gene content and origins of clones.

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Conflicts of Interest

The authors declare that they all have seen the final submitted draft and that there are no conflicts of interest.

Ethical Statement

This study only analyzed bacterial isolates stored after isolation from patients. Any detail about the patent name or privacy was not available to us because we did not work with the patients directly, and hence, would be exempted from criteria under human subject research, ethical approval.

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